100. *Experiments on the Synthesis of Purine Nucleosides. Part I. Model Experiments on the Synthesis of 9-Alkylpurines.*

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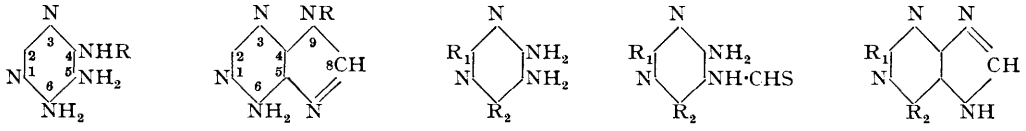
A series of model experiments is described, in which a hypothetical route to the synthesis of purine nucleosides is examined. The conversion of 4-amino-5-thioformamidopyrimidines into purines can be carried out by boiling in aqueous, pyridine or quinoline solution. As anticipated on theoretical grounds, similar treatment of 6-*amino*-4-*methylamino*-5-*thioformamido*-2-*methylthiopyrimidine* yields exclusively 2-*methylthio*-9-*methyladenine*, indicating a convenient route to the synthesis of 9-substituted 6-aminopurines. In the course of the work, a number of other new pyrimidine and purine derivatives have been prepared.

The importance of the purine nucleosides, not only as components of nucleic acids but also in the composition of enzyme systems concerned in vital processes, has become increasingly clear in recent years. Adenosine (9-*d*-ribofuranosidoadenine) (II; R = *d*-ribofuranose) is known to form an essential part of a number of co-enzymes concerned in the transfer of hydrogen and phosphate in biological systems, and we have for some time been engaged in experiments designed to test the possibility of a synthesis of this and other 9-glycosidopurines. A successful outcome of these experiments might, by rendering such substances available, further our knowledge of the relation between chemical structure and co-enzyme function.

Although it is now more than thirty years since the first purine nucleoside was isolated, no synthesis of a naturally occurring compound of this class has yet been reported, and it is only recently that the location of the sugar residue in them at N_9 has been established by a study of their ultra-violet absorption spectra (Gulland and Holiday, J., 1936, 765). Fischer and Helferich (*Ber.*, 1914, **47**, 210) prepared *d*-glucosides of adenine, guanine, and hypoxanthine from the reaction product of acetobromoglucose and trichloropurine silver, and, more recently, Gulland and Story (J., 1938, 259) have shown by spectroscopic methods, that the glucose residue in these compounds is in the N_9 position. The glycosidic grouping in these compounds is, however, of the pyranose type, whereas the natural nucleosides have a furanose structure. Although the synthetic method of these authors could doubtless be applied to the synthesis of, say, adenosine, it is rather limited in scope for our purposes, which involve the study of a wide range of adenosine analogues. Gulland and his collaborators have also investigated two other possible synthetic routes, either of which would provide unequivocal proof of the position of the glycosidic residue in the products. In the first of these (Gulland and Macrae, J., 1933, 662) an unsuccessful attempt was made to obtain theophylline 9-*d*-glucoside by condensing 1 : 3-dimethyluramil with tetra-acetylglucose *iso*thiocyanate; in the second (Allsebrook, Gulland, and Story, J., 1942, 232) efforts to synthesise a 9-glucosidopurine from an *N*-glucosidoglyoxaline failed owing to the inaccessibility of the appropriate glyoxaline derivative.

In our approach to the problem we envisaged a synthesis involving the preparation of a 5 : 6-diamino-4-glycosidaminopyrimidine (*e.g.*, I; R = *d*-ribofuranose), followed by conversion of this into the required 9-glycosidoadenine (*e.g.*, II; R = *d*-ribofuranose). This method is analogous to the well-known Traube synthesis of adenine (*Annalen*, 1904, **331**, 64), in which a compound of type (I; R = H) is heated with formic acid, the 5-formamido-derivative first produced cyclising at higher temperatures by loss of water. Owing to its ease of hydrolysis a 9-glycosidoadenine would in all probability be destroyed under such drastic reaction conditions if indeed it were formed at all. We therefore directed our attention in the first instance to the elaboration of a milder cyclisation procedure, employing as model substances sugar-free pyrimidine derivatives.

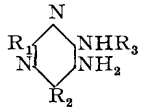


(I.) (II.) (III.) (IV.) (V.)

A possible method for converting 4 : 5-diaminopyrimidines into purines under mild conditions was suggested by the observation of Todd, Bergel, and Karimullah (J., 1936, 1557) that 4 : 5-diamino-6-methylpyrimidine (III; R_1 = H, R_2 = Me) on treatment with aqueous potassium dithioformate yielded 4-amino-5-thioformamido-6-methylpyrimidine (IV; R_1 = H, R_2 = Me), which when heated above its m. p. readily evolved hydrogen sulphide, giving 6-methylpurine (V; R_1 = H, R_2 = Me). It has now been found that the conversion of 4-amino-5-thioformamidopyrimidines into purines can be brought about readily by boiling their solutions in water or anhydrous organic solvents such as pyridine or quinoline until evolution of hydrogen sulphide ceases; the generality of this method has been tested by application to the synthesis of a number of purines. On the whole the use of anhydrous organic solvents in the reaction is recommended, since in certain cases boiling with water leads to the production of considerable amounts of 5-formamidopyrimidine. 4 : 5-Diamino-6-hydroxy-2-methylpyrimidine (III; R_1 = Me, R_2 = OH), treated with aqueous sodium dithioformate, yielded the corresponding 5-*thioformamido*-compound (IV; R_1 = Me, R_2 = OH), convertible into 6-hydroxy-2-methylpurine (V; R_1 = Me, R_2 = OH) on boiling with quinoline.

4 : 5 : 6-*Triamino*-2-*methylthiopyrimidine* (III; R_1 = SMe, R_2 = NH_2), prepared from 4 : 6-diamino-2-methylthiopyrimidine by treatment with nitrous acid and reduction of the resulting 5-nitroso-derivative

with ammonium sulphide, gave with sodium dithioformate 4 : 6-*diamino*-5-*thioformamido*-2-*methylthiopyrimidine* (IV; R_1 = SMe, R_2 = NH_2), cyclised to 2-*methylthioadenine* (V; R_1 = SMe, R_2 = NH_2). In similar fashion 4 : 6-*diamino*-5-*thioformamido*-2-*methylpyrimidine* (IV; R_1 = Me, R_2 = NH_2) yielded 2-*methyladenine* (V; R_1 = Me, R_2 = NH_2). The thioformamido-compound was in this case prepared from 4 : 6-dihydroxy-2-methylpyrimidine. The latter, on heating with phosphoryl chloride, gave 4 : 6-*dichloro*-2-*methylpyrimidine*, aminated successively to 4-chloro-6-amino-2-methylpyrimidine and 4 : 6-diamino-2-methylpyrimidine. Treatment of this diamine with dilute hydrochloric acid and sodium nitrite gave the 5-nitroso-derivative, which was reduced with ammonium sulphide and the crude product converted directly into (IV; R_1 = Me, R_2 = NH_2) by means of aqueous sodium dithioformate.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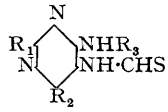
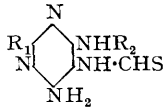

(VII.)


(VIII.)

We next applied the procedure described to the synthesis of 9-alkylpurines. On heating a quinoline solution of 4-*methylamino*-5-*thioformamido*-2-*hydroxy*-6-*methylpyrimidine* (VII; R_1 = OH, R_2 = R_3 = Me), ring-closure occurred readily, giving 2-*hydroxy*-6 : 9-*dimethylpurine* (VIII; R_1 = OH, R_2 = R_3 = Me). The ready cyclisation in this case was not unexpected, since Johns (*J. Biol. Chem.*, 1911, **9**, 161) observed that 5-amino-6-methylamino-2-hydroxypyrimidine reacted with formic acid to give the corresponding purine even more readily than 4 : 5-diamino-2-hydroxypyrimidine.

For the preparation of a 9-alkyladenine by this method a 6-amino-4-alkylamino-5-thioformamidopyrimidine is required and theoretically ring closure might occur in two ways, giving either a 9-alkylpurine (X) or a 6-alkylaminopurine (XI).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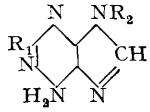
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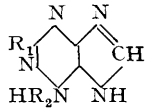
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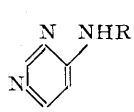
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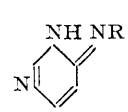
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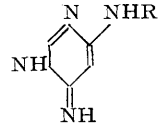
(XIV.)

It seemed to us probable that the ring closure would in fact lead to the production of the 9-alkylpurine (X). Our experience of the behaviour of pyrimidine derivatives suggested that a 4- or 6-alkylaminopyrimidine would tend to react more in the form (XII; R = alkyl) and less in the tautomeric iminodihydropyrimidine form (XIII ; R = alkyl) than would be the case with a 4- or 6-aminopyrimidine (XII and XIII ; R = H), *i.e.*, a 4-alkylamino-6-aminopyrimidine would react as if it had structure (XIV). In this case cyclisation of (IX) by loss of hydrogen sulphide would be expected to lead to (X) rather than (XI). The ready cyclisation of 4-alkylamino-5-thioformamidopyrimidines already noted accords with this view.

In order to decide which type of reaction did, in fact, occur, we investigated the behaviour of 6-*amino*-4-*methylamino*-5-*thioformamido*-2-*methylthiopyrimidine* (IX; R_1 = SMe, R_2 = Me). 4-Chloro-6-amino-2-methylthiopyrimidine, reacting with methylamine, gave 6-*amino*-4-*methylamino*-2-*methylthiopyrimidine*, which was nitrosated, reduced, and the crude product thioformylated to give the required (IX; R_1 = SMe, R_2 = Me). When an aqueous solution of this compound was boiled till hydrogen sulphide evolution ceased, only one product could be isolated. This was identical with the product obtained when 2-methylthioadenine was methylated under the conditions described by Krüger (*Z. physiol. Chem.*, 1894, **18**, 434) for the conversion of adenine into 9-methyladenine; it is therefore to be regarded as the expected 2-*methylthio*-9-*methyladenine* (X; R_1 = SMe, R_2 = Me). In accordance with this view of its structure it underwent deamination with nitrous acid at 60°, giving 6-*hydroxy*-2-*methylthio*-9-*methylpurine* (2-*methylthio*-9-*methylhypoxanthine*). No evidence for the formation of a substance of type (XI) could be obtained.

If, as might be anticipated, a 4-glycosidamino-group in the pyrimidine nucleus behaves in similar fashion to a 4-methylamino-group, then the synthetic method described should be applicable to the preparation of 9-glycosidopurines of the adenosine type, since we have established that the cyclisation stage can be accomplished by boiling solutions of the 5-thioformamido-compounds in anhydrous solvents such as pyridine, thus avoiding the considerable risk of hydrolysis which would attend boiling in aqueous solution.

Experimental.

The sodium dithioformate used throughout was the crystalline hexahydrate, $H{\cdot}CS{\cdot}SNa,6H_2O$.

4-*Amino*-5-*thioformamido*-6-*hydroxy*-2-*methylpyrimidine* (IV; R_1 = Me, R_2 = OH).—4 : 5-Diamino-6-hydroxy-2-methylpyrimidine (25 g.) (Traube, *Annalen*, 1923, **432**, 287) was dissolved in water (1 l.) at 65°, aqueous sodium dithioformate (50 g. in 350 c.c. of water) added, and the solution cooled rapidly. A small amount of the pyrimidine separated but subsequently redissolved. After standing overnight, the *thioformyl* derivative which had separated was collected and recrystallised from warm water, forming colourless plates which decomposed slowly above 260° without melting (Found : C, 39·3; H, 4·2; N, 30·7. $C_6H_8ON_4S$ requires C, 39·1; H, 4·3; N, 30·4%). Yield, quantitative.

6-*Hydroxy*-2-*methylpurine* (2-*Methylhypoxanthine*) (V; R_1 = Me, R_2 = OH).—The above thioformamido-compound (3 g.) was refluxed with quinoline (30 c.c.) for ½ hour. Dissolution was followed by evolution of hydrogen sulphide and the

purine gradually separated. The solid was collected and recrystallised from hot water, forming colourless needles (2·7 g.) which decomposed above 360° [Found in material dried at room temperature over calcium chloride: C, 38·3; H, 5·5; N, 30·7; loss at 140° (P_2O_5), 20·0. Calc. for $C_6H_6ON_4,2H_2O$: C, 38·7; H, 5·4; N, 30·2; loss ($2H_2O$), 19·6%]. The properties of the compound agreed with those given by Traube (*Annalen*, 1923, **432**, 287) for 2-methylhypoxanthine.

4 : 5 : 6-*Triamino-2-methylthiopyrimidine* (II; R_1 = SMe, R_2 = NH_2).—A solution of 4 : 6-diamino-2-methylthiopyrimidine (10 g.) (Wheeler and Jamieson, *Amer. Chem. J.*, 1904, **32**, 349) in a mixture of water (500 c.c.) and glacial acetic acid (28 c.c.) was cooled in ice, and a similarly cooled solution of sodium nitrite (9·6 g. in 100 c.c. of water) added. After standing for 2 hours at 0°, the light blue nitroso-compound was collected, washed, suspended in water (100 c.c.), and reduced by adding ammonium sulphide solution (41 c.c. of 17% aqueous ammonia saturated with hydrogen sulphide at 0°). After the mixture had been shaken for 2 hours, the greenish precipitate of 4 : 5 : 6-*triamino-2-methylthiopyrimidine* was collected and recrystallised from water (charcoal), forming long needles (5 g.), m. p. 182° (Found: C, 34·9; H, 5·2. $C_5H_9N_5S$ requires C, 35·1; H, 5·3%).

4 : 6-*Diamino-5-thioformamido-2-methylthiopyrimidine* (IV; R_1 = SMe, R_2 = NH_2).—Sodium dithioformate (5·8 g.) was added to a solution of the above triamino-compound (3·7 g.) in water (450 c.c.) at 40°. After standing overnight, the *thioformyl* derivative was collected. It crystallised from hot water in faintly yellow, silky needles (3·5 g.), m. p. 235° (decomp.) (rapid heating) (Found: C, 33·4; H, 4·4; N, 32·5. $C_6H_9N_5S_2$ requires C, 33·5; H, 4·2; N, 32·6%).

2-*Methylthioadenine* (V; R_1 = SMe, R_2 = NH_2).—The above thioformamido-compound (3·4 g.) was boiled with water (200 c.c.) for 36 hours. The *purine* separated on cooling and was recrystallised from water (charcoal), forming colourless silky needles (2 g.), m. p. 290° (decomp.) (Found: C, 35·7; H, 4·6; N, 35·5. $C_6H_7N_5S,H_2O$ requires C, 36·2; H, 4·5; N, 35·2%). The same product was obtained on refluxing the thioformamido-compound with quinoline for 20 minutes.

4 : 6-*Dichloro-2-methylpyrimidine*.—A mixture of 4 : 6-dihydroxy-2-methylpyrimidine (20 g.) (Dox and Yoder, *J. Amer. Chem. Soc.*, 1922, **44**, 361) and phosphoryl chloride (140 c.c.) was refluxed till evolution of hydrogen chloride ceased (*ca.* 2 hours). The excess of phosphoryl chloride was removed in a vacuum, and the residual oil poured on ice. The solid product was collected, washed with water, and dried in a desiccator. After sublimation in a vacuum 4 : 6-*dichloro-2-methylpyrimidine* formed colourless needles, m. p. 48—49° (yield, 75%) (Found: Cl, 43·1. $C_5H_4N_2Cl_2$ requires Cl, 43·6%). This compound was first prepared by Mr. A. Parkinson in these laboratories in the course of other investigations.

4-*Chloro-6-amino-2-methylpyrimidine*.—The above dichloro-compound (1 g.) was heated in a sealed tube with saturated methyl-alcoholic ammonia (6 c.c.) at 130° during 3 hours. After removal of the solvent and recrystallisation from methanol the product formed colourless needles (0·5 g.), m. p. 190—191° (Found: C, 42·1; H, 4·3; N, 29·4; Cl, 25·4. Calc. for $C_5H_6N_3Cl$: C, 41·9; H, 4·2; N, 29·3; Cl, 24·8%). Földi, von Fodor, Demjén, Szekeres, and Halmos (*Ber.*, 1942, **75**, 755) give m. p. 189°.

4 : 6-*Diamino-2-methylpyrimidine*.—4 : 6-Dichloro-2-methylpyrimidine (1·6 g.) was heated in a sealed tube with saturated methyl-alcoholic ammonia (12 c.c.) at 200° for 4 hours. The cooled solution was filtered from ammonium chloride and evaporated, and the residue extracted with boiling *n*-butanol. The extract was evaporated under reduced pressure, and the residue dissolved in alcohol, shaken with a little aluminium oxide, filtered, and concentrated to small bulk. The *diamino*-compound separated on cooling in colourless needles, m. p. 294—295° (sealed tube). Yield, 75% (Found: C, 48·6; H, 6·7; N, 44·7. $C_5H_8N_4$ requires C, 48·5; H, 6·5; N, 45·1%). The substance was readily soluble in water and alcohol and formed a *picrate* crystallising from water in long yellow needles which on heating decomposed above 250° (Found: C, 37·5; H, 3·2; N, 27·6. $C_5H_8N_4,C_6H_3O_7N_3$ requires C, 37·3; H, 3·1; N, 27·7%).

4 : 6-*Diamino-5-thioformamido-2-methylpyrimidine* (IV; R_1 = Me, R_2 = NH_2).—To a solution of 4 : 6-diamino-2-methylpyrimidine (2 g.) in 3N-hydrochloric acid (25 c.c.) at 0°, ice-cold sodium nitrite (1·5 g. in 10 c.c. of water) was added. After 1 hour at 0° the pale blue nitroso-compound was collected; it showed no m. p. below 300°. Reduction was effected by suspending it in an ice-cold solution of ammonium sulphide (from 80 c.c. of 14% aqueous ammonia saturated at 0° with hydrogen sulphide) and shaking for 2 hours. The brownish solution was evaporated to dryness under reduced pressure, and the residue extracted with warm water. The extract was filtered, sodium dithioformate (6 g.) added, and the mixture kept overnight. The precipitated *thioformamido*-compound crystallised from hot water (charcoal) in colourless needles which had no definite m. p. (yield, 35%) (Found: C, 39·2; H, 4·6; N, 38·0; S, 17·1. $C_6H_9N_5S$ requires C, 39·4; H, 4·9; N, 38·2; S, 17·5%).

2-*Methyladenine* (V; R_1 = Me; R_2 = NH_2).—The above thioformyl derivative (10 g.) was refluxed with quinoline (100 c.c.) for 30 mins., during which time it passed into solution with evolution of hydrogen sulphide and the *purine* rapidly crystallised. After cooling, the product was collected, washed with alcohol, and purified by dissolution in dilute hydrochloric acid and precipitation with ammonia (yield, 75%). It was sparingly soluble in hot water, from which it crystallised in tiny plates which did not melt below 300° (Found: C, 48·3; H, 4·7; N, 47·1. $C_6H_7N_5$ requires C, 48·3; H, 4·7; N, 47·0%). The cyclisation was very much slower in pyridine solution. The *picrate* crystallised from water in yellow needles decomposing above 250° (Found: C, 38·0; H, 2·6; N, 29·6. $C_6H_7N_5,C_6H_3O_7N_3$ requires C, 37·7; H, 2·6; N, 30·2%).

4-*Methylamino-5-thioformamido-2-hydroxy-6-methylpyridimine* (VII; R_1 = OH, R_2 = R_3 = Me).—Thioformylation of 5-amino-4-methylamino-2-hydroxy-6-methylpyrimidine (0·25 g.) (Johns, *J. Biol. Chem.*, 1912, **11**, 393) in the normal manner with sodium dithioformate (0·25 g.) yielded a *product* crystallising from water in colourless prisms (0·22 g.) which on heating evolved hydrogen sulphide but did not melt below 300° (Found: N, 28·0. $C_7H_{10}ON_4S$ requires N, 28·3%).

2-*Hydroxy*-6 : 9-*dimethylpurine*.—The above thioformyl derivative (3·2 g.), refluxed with quinoline (50 c.c.) for 20 mins. and worked up in the usual manner, gave a *product* crystallising from water (charcoal) in needles which did not melt below 300° (Found: C, 51·0; H, 4·9; N, 34·6. $C_7H_8ON_4$ requires C, 51·2; H, 4·9; N, 34·1%).

6-*Amino-4-methylamino-2-methylthiopyrimidine* (VI; R_1 = SMe, R_2 = NH_2, R_3 = Me).—4-Chloro-6-amino-2-methylthiopyrimidine (1 g.) (Johnson and Johns, *Amer. Chem. J.*, 1905, **34**, 183) was heated in a sealed tube with aqueous methylamine (1·4 g. of 33%) at 100° for 3 hours. The crystalline solid which separated was recrystallised from water, forming colourless needles (0·75 g.), m. p. 143—144° (Found: C, 42·5; H, 5·8; N, 32·6; S, 18·3. $C_6H_{10}N_4S$ requires C, 42·4; H, 5·9; N, 33·0; S, 18·8%).

6-*Amino-4-methylamino-5-thioformamido-2-methylthiopyrimidine* (IX; R_1 = SMe, R_2 = Me).—6-Amino-4-methylamino-2-methylthiopyrimidine (4 g.) was dissolved in dilute acetic acid (50 c.c. of 10%) and treated at 0° with a concentrated aqueous solution of sodium nitrite (2 g.). After standing overnight, the pale blue nitroso-compound was collected (4 g., m. p. 236°), suspended in ammonium sulphide solution (from 20 c.c. of 17% aqueous ammonia saturated with hydrogen sulphide at 0°), and warmed to 35°; it then dissolved and a yellow solid began to separate. When no more separated, the mixture was evaporated under reduced pressure, and the residue extracted repeatedly with boiling water. To the combined extracts, sodium dithioformate (8 g.) was added and after standing overnight the *thioformyl* derivative was collected. Recrystallised from water at 70° (charcoal), it formed colourless needles (3·5 g.), m. p. 185—186° with evolution of hydrogen sulphide (Found: C, 36·7; H, 5·2; N, 30·4. $C_7H_{11}N_5S_2$ requires C, 36·6; H, 5·0; N, 30·6%).

2-*Methylthio*-9-*methyladenine* (X; R_1 = SMe, R_2 = Me).—(a) *From the above thioformamido-compound.* The substance (IX; R_1 = SMe, R_2 = Me) (3 g.) was boiled with water (100 c.c.) for 5 hours, separated solid brought into solution by adding more water, and after boiling with charcoal for a short time the mixture was filtered and cooled. Colourless needles

of 2-*methylthio*-9-*methyladenine* separated (Found: C, 43·2; H, 4·6; N, 36·4. $C_7H_9N_5S$ requires C, 43·0; H, 4·6; N, 35·9%). The product had m. p. 261—262°, undepressed on admixture with a specimen prepared by route (b) below. From the crystallisation mother-liquors further quantities of the same compound were obtained, the yield of purified product being 94%; no other substance was detected. Similar results were obtained when the thioformamido-compound was refluxed with pyridine or quinoline.

(b) *From* 2-*methylthioadenine*. 2-Methylthioadenine (0·36 g.) was refluxed with alcoholic sodium hydroxide (0·08 g. in 30 c.c. of alcohol) and methyl iodide (0·32 g.) for 1 hour and kept overnight at room temperature. The 2-methylthio-9-methyladenine which separated crystallised from water in colourless needles, m. p. 262—263°.

Deamination of 2-*Methylthio*-9-*methyladenine*.—The purine [0·5 g., prepared by method (a) above] was dissolved in boiling water (200 c.c.) containing acetic acid (3 c.c.); after cooling to 60—65°, barium nitrite (2·5 g.) was added, and the solution maintained at this temperature for 45 mins. A slight excess of ammonia was added, and the solution evaporated to one-third its bulk and cooled. The product which separated was dissolved in dilute hydrochloric acid, and the solution decolourised with charcoal and neutralised to litmus with sodium hydroxide. On adding acetic acid (6 c.c.) to the cooled solution, 6-*hydroxy*-2-*methylthio*-9-*methylpurine* (0·3 g.) crystallised in fine needles, m. p. 332° (decomp.) (Found: C, 42·5; H, 4·25; N, 28·5. $C_7H_8ON_4S$ requires C, 42·9; H, 4·1; N, 28·6%).

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